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Phylogeny and host-specificity of European seed beetles (Coleoptera, Bruchidae), new insights from molecular and ecological data

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Abstract

We used partial sequences of three mitochondrial genes (12S rRNA, cytochrome *b*, and cytochrome *c* oxidase subunit I) to reconstruct the phylogeny of European seed beetles (Bruchidae) belonging to the genera *Bruchus* Linnaeus and *Bruchidius* Schilsky. Adult beetles examined in this study were obtained from larvae bred from seeds directly collected in the field. Parsimony, maximum likelihood, and Bayesian inference were used to infer phylogenetic relationships among species. Both genera, *Bruchidius* and *Bruchus*, formed monophyletic groups in all analyses. Our results were partially in discrepancy with existing taxonomic groups (Borowiec, 1987). Critical analysis of relationships among taxa, and exhaustive review of host-plants data highlight the very high level of specialization of these seed beetles. Phylogenetically related insects were associated with host-plants belonging to the same botanical tribes.

Keywords: 12S rRNA; Bayesian inference; Bruchidae; Cytochrome *c* oxidase subunit I; Cytochrome *b*; Host-specificity; Maximum likelihood; Parsimony; Phylogenetic inference; Taxonomy

1. Introduction

Complex interactions between phytophagous insects and their host-plants have given rise to numerous studies. Two clear patterns have emerged from these studies: (1) phytophagous clades are far more diversified than their non-phytophagous sister clades (Mitter et al., 1988), (2) phytophagous insects show a strong trend towards specialization and are associated with specific plant taxa and tissues (Marvaldi et al., 2002). Most studies deal with Lepidoptera and phytophagous Coleoptera (Chrysomeloidea and Curculionoidea), which represent the majority of phytophagous insect species (i.e., 135,000 species; Lawrence, 1982), and 141,000 species of Lepidoptera (Powell et al., 1999). The understanding of the mechanisms underlying these evolutionary patterns requires phylogenetic assumptions for many groups of phytophagous insects and the integration of all available ecological data. Ehrlich and Raven (1964) were the first to formulate an explanatory assumption based on the study of associations between Lepidoptera and their host-plants. For them, a coevolution process exists between phytophagous insects and their host-plants in the form of an arms race which stimulates, in a reciprocal way, the diversification of the two groups: the plants escape from the phytophagous insects by developing toxic secondary compounds, and the insects able to detoxify these compounds (evolutionary key-innovations) diversify on plants having similar secondary compounds. In some cases, the diversification of the insects can follow the radiation of their host-plants, and thus yields congruent phylogenies (Farrell and Mitter, 1990; Silvain and Delobel, 1998). Within the superfamily Chrysomeloidea, the family Bruchidae exhibits ecological characteristics that strengthen their usefulness in evolutionary studies. The larval stages of bruchids develop inside seeds. Often, mating occurs near the host plant seeds from which adults have emerged. Bruchids are generally oligophagous

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Table 1
Material examined in this study

Genus Species	Locality[a]	Host-plant[b] (Family, Tribe)[c]	GenBank Accession No. 12S rRNA	 COI	 Cyt *b*
Bruchidius					
biguttatus (Olivier, 1795)	Gard (Fr.)	*Cistus albidus* (**C**)	AY390639	AY390671	AY390703
bimaculatus (Olivier, 1795)	Haute Corse (Fr.)	*Medicago marina* (**F**: Tri)	AY390640	AY390672	AY390704
caninus (Kraatz, 1869)	Haute Corse (Fr.)	*Astragalus hamosus* (**F**: Gal)	AY390641	AY390673	AY390705
cinerascens (Gyllenhal, 1833)	Essone (Fr.)	*Eryngium campestre* (**Ap**)	AY390642	AY390674	AY390706
dispar (Gyllenhal, 1833)	Rhône (Fr.)	*Trifolium medium* (**F**: Tri)	AY390643	AY390675	AY390707
fulvicornis (Motschulsky, 1874)	Corse du Sud (Fr.)	*Trifolium vesiculosum* (**F**: Tri)	AY390644	AY380676	AY390708
lividimanus (Gyllenhal, 1833)	Rhône (Fr.)	*Cytisus scorparius* (**F**: Gen)	AY390645	AY390677	AY390709
marginalis (Fabricius, 1776)	Gard (Fr.)	*Astragalus monspessulanus* (**F**: Gal)	AY390646	AY390678	AY390710
nanus (Germar, 1824)	Basilicata (It.)	*Medicago orbicularis* (**F**: Tri)	AY390647	AY390679	AY390711
pauper (Boheman, 1829)	Corse du Sud (Fr.)	*Ornithopus compressus* (**F**: Lot)	AY390648	AY390680	AY390712
picipes (Germar, 1824)	Corse du Sud (Fr.)	*Trifolium angustifolium* (**F**: Tri)	AY390649	AY390681	AY390713
pusillus (Germar, 1824)	Gard (Fr.)	*Hippocrepis emerus* (**F**: Lot)	AY390650	AY390682	AY390714
pygmaeus (Boheman, 1833)	Gard (Fr.)	*Trifolium angustifolium* (**F**: Trif)	AY390651	AY390683	AY390715
seminarius (Linnaeus, 1767)	Vaucluse (Fr.)	*Lotus maritimus* (**F**: Lot)	AY390652	AY390684	AY390716
sericatus (Germar, 1824)	Corse du Sud (Fr.)	*Trifolium angustifolium* (**F**: Trif)	AY390653	AY390685	AY390717
trifolii (Motschulsky, 1874)	Baharîya (Eg.)	*Trifolium alexandrinum* (**F**: Trif)	AY509806	AY509809	AY509812
unicolor (Olivier, 1795)	Vaucluse (Fr.)	*Onobrychis sativa* (**F**: Hed)	AY390654	AY390686	AY390718
varipictus (Motschulsky, 1874)	Haute Corse (Fr.)	*Medicago murex* (**F**: Tri)	AY390657	AY390689	AY390720
villosus (Fabricius, 1792)	Vaucluse (Fr.)	*Cytisophyllum sessilifolium* (**F**: Gen)	AY390655	AY390687	AY390719
pr. varius (Olivier, 1795)	Corse du Sud (Fr.)	*Trifolium angustifolium* (**F**: Tri)	AY390656	AY390688	
Bruchus					
affinis Frölich, 1799	Haute Corse (Fr.)	*Lathyrus latifolius* (**F**: Vic)	AY390658	AY390690	AY390721
atomarius (Linnaeus, 1761)	Vaucluse (Fr.)	*Lathyrus macrorhyzus* (**F**: Vic)	AY390659	AY390691	AY390722
brachialis Fahraeus, 1839	Haute Corse (Fr.)	*Vicia villosa* (**F**: Vic)	AY390660	AY390692	AY390723
laticollis Boheman, 1833	Vaucluse (Fr.)	*Lathyrus aphaca* (**F**: Vic)	AY509807	AY509810	AY509813
loti Paykull, 1800	Oise (Fr.)	*Lathyrus pratensis* (**F**: Vic)	AY390661	AY390693	AY390724
luteicornis Illiger, 1794	Vaucluse (Fr.)	*Vicia sativa* (**F**: Vic)	AY390662	AY390694	AY390725
rufimanus Boheman, 1833	Vaucluse (Fr.)	*Vicia pannonica* (**F**: Vic)	AY390663	AY390695	AY390726
rufipes Herbst, 1783	Haute Corse (Fr.)	*Vicia sativa* (**F**: Vic)	AY390664	AY390696	AY390727
tristiculus Fahraeus, 1839	Vaucluse (Fr.)	*Lathyrus cicera* (**F**: Vic)	AY390666	AY390698	AY390729
tristis Boheman, 1833	Vaucluse (Fr.)	*Lathyrus cicera* (**F**: Vic)	AY390667	AY390699	AY390730
viciae Olivier, 1795	Basilicata (It.)	*Lathyrus sphaericus* (**F**: Vic)	AY509808	AY509811	AY509814
Pachymerus					
cardo (Fahraeus, 1839)	French Guyana	*Elais sp.* (**Ar**)	AY390636	AY390668	AY390700
Paleoacanthoscelides					
gilvus (Gyllenhal, 1839)	Vaucluse (Fr.)	*Onobrychis sativa* (**F**: Hed)	AY390638	AY390670	AY390702
Spermophagus					
sp.	Gard (Fr.)		AY390637	AY390669	AY390701

[a] Egypt (Eg.) France (Fr.), Italy (It.).
[b] We only figured the host-plant species from which the sequenced individual has been reared.
[c] Host-plants systematic was abbreviated as follows: **Ap** (Apiaceae), **Ar** (Arecaceae), **C** (Cistaceae), **F** (Fabaceae), Gal (Galegeae), Gen (Genisteae), Hed (Hedysereae), Lot (Loteae), Tri (Trifolieae), and Vic (Vicieae).

(they feed on several species belonging to a single host family) or monophagous (they feed on a single species) (Borowiec, 1987). Most bruchid species use Fabaceae (Leguminoseae) as host-plants (Johnson, 1981). This strong host-specificity has been correlated with the diversity of plant-defense mechanisms and particularly with the presence of toxic secondary compounds in seeds (Janzen, 1981). Bruchidae constitute a very good model for plant–insect interaction studies (Jermy and Szentesi, 2003; Johnson, 1981): seed sampling in the field and monitoring of adult emergences give the opportunity to accurately identify insect–plant associations, and thus to highlight possible coevolutionary processes between insects and their hosts. Moreover, this group of beetles is of great interest as it contains several pest species of economic importance in both temperate and tropical areas, such as *Acanthoscelides obtectus* (Say), *Bruchus pisorum* Linnaeus, *Callosobruchus maculatus* (Fabricius) or *Caryedon serratus* (Olivier). Thirty-one European species of *Bruchus* and *Bruchidius*, which belong to the

Bruchinae, one of the 6 subfamilies of Bruchidae, were studied. The genus *Bruchus* is morphologically well defined. Several characters distinguish it from other Bruchidae (Borowiec, 1987): (1) pronotum with lateral denticle, (2) unique structure of the mid tibia in male, and (3) male genitalia. In contrast, the genus *Bruchidius* has not been defined on the basis of synapomorphies. It artificially brings together a great number of Old World species. According to Johnson (1981), this genus is probably paraphyletic and should be divided into smaller monophyletic genera. *Bruchus* species are found in the Paleartic Region (with the exception of introduced species) and are essentially European (Borowiec, 1987). Regarding European species of *Bruchidius*, we have no evidence of their monophyly though they form an apparently homogeneous group compared to African species. Three outgroups have been chosen: another representative of the subfamily Bruchinae, *Paleoacanthoscelides gilvus* (Gyllenhal); a representative of the subfamily Amblycerinae, belonging to the genus *Spermophagus*; and *Pachymerus cardo* (Fahraeus), a representative of the primitive subfamily Pachymerinae. At the generic level, these beetles show little external morphological differentiation and characters on the male genitalia have not been studied in a phylogenetic perspective until now. Therefore molecular phylogenetics may be a way to obtain useful phylogenetic information. We used partial sequences of three mitochondrial genes to elucidate phylogenetic relationships among species. Our objective is to test the monophyly of the two genera, clarify the systematics of these species, and test the validity of several taxonomic groups established by Borowiec (1988). We also mapped host-plant preferences on phylogenetic trees in order to highlight evolutionary patterns between insects and host-plants. A critical review of French bruchid host-plants (Delobel and Delobel, 2003) will help us testing the assumption according to which insect species consuming phylogenetically close plants are related. We are also particularly interested in the phylogenetic position of species feeding on plants outside the Fabaceae, in order to achieve a better understanding of host-shift mechanisms.

2. Materials and methods

2.1. Taxon sampling

Seeds were collected in the field on potential host-plants for three years (from 2000 to 2002). Table 1 indicates the species, localities, and host-plants of the species analysed. Although the *Bruchidius trifolii* specimen was collected in Egypt, we included it in this study because this species presents a circummediterranean distribution. Adults were obtained by rearing larvae infesting these seeds, with the exception of *Spermophagus* and *Pachymerus cardo* specimens. The latter was collected in 1997 in French Guyana. Adults were fixed and stored in 100% ethanol until used. For species identification, we mainly used male genitalia in association with several external morphological key-characters (Anton, 1998, 2001; Borowiec, 1988). Specimens corresponding to this study are kept in the IRD collection of the Muséum National d'Histoire Naturelle (45 rue Buffon, Paris).

2.2. DNA extraction and polymerase chain reaction

DNA was extracted from whole individuals except for the Pachymerinae representative for which we only used antennae and hind legs. After grinding the samples in a PBS solution, we followed the protocol recommended by the Qiagen DNeasy tissue kit (Qiagen GmbH, Germany). Amplification of partial sequences of the three mitochondrial genes was accomplished by standard PCR amplification using primers listed in Table 2. For the amplification of the 3′ half of 12S rRNA gene we used primers SR-J-14233 and SR-N-14588. Amplification of 1 kb gene region of the COI gene was accomplished by amplifying two overlapping

Table 2
Primers used in the amplification of 12S rRNA, COI, and Cyt *b*

Gene	Name of primer[a,b]	Sequence of primer	Max Length (bp) of sequenced product
12S rRNA	SR-J-14233 (f)	5′-AAGAGCGACGGGCGATGTGT-3′	401
	SR-N-14588 (r)	5′-AAACTAGGATTAGATACCCTATTAT-3′	
COI	C1-J-1751 (f)	5′-GGATCACCTGATATAGCATTCCC-3′	1018
	C1-N-2191 (r)	5′-CCCGGTAAAATTAAAATATAAACTTC-3′	
	Tonya (f)	5′-GAAGTTTATATTTTAATTTTACCGGG-3′	
	Hobbes (r)	5′-AAATGTTGNGGRAAAAATGTTA-3′	
Cyt *b*	CP1 (f)	5′-GATGATGAAATTTTGGATC-3′	782
	CB-J-10933 (f)	5′-TATGTACTACCATGAGGACAAATATC-3′	
	CB-N-11367 (r)	5′-ATTACACCTCCTAATTTATTAGGAAT-3′	

[a] All the primers except Tonya, Hobbes (Monteiro and Pierce, 2001), and CP1 followed Simon et al. nomenclature (1994).
[b] Forward reading (f), reverse reading (r).

fragments of about 500 bp using primer C1-J-1751 in combination with C1-N-2191 and primer Tonya in combination with Hobbes. For the amplification of fragment of the Cyt *b* gene we used primers CP1or CB-J-10933 in combination with CB-N-11367. Typical PCRs were prepared in 50 µl volumes using 2 U *Taq*-polymerase and 2 µl of genomic DNA at 2.5 mM $MgCl_2$ and 1 mM dNTP concentration. PCR cycling conditions started with an initial 5 min denaturing step at 92 °C followed by 35 amplification cycles of 1 min denaturing at 92 °C, 30 s annealing at 52 °C, 1 min extension at 72 °C, and final extension step at 72 °C for 10 min. The cycling conditions for COI were the same but 40 amplification cycles were necessary with 1 min annealing at 47 °C. For Cyt *b* 37 cycles were used with 1 min 30 s annealing at 48 °C and 2 min extension at 72 °C. Reactions were then cooled to 4 °C until removal. PCR products were purified by using QIAquick PCR purification kit

Table 3
Base composition and sequence variation for 12S rRNA, COI, and Cyt *b*

Gene		A%	C%	G%	T%	Total sites	Variable sites	Informative sites	Mean/maximum pairwise sequence divergence (%)	
12S rRNA		39.87	14.10	9.13	36.89	406	128	85	7.58 ± 2.51	15.18
COI		29.15	16.47	16.57	37.78	1018	395	321	13.21 ± 2.48	19.60
	1st Pos.	29.18	15.60	29.43	25.77	339	72	47		
	2nd Pos.	16.07	23.95	16.48	43.49	339	23	6		
	3rd Pos.	42.20	9.89	3.82	44.07	340	300	268		
Cyt *b*		30.28	17.32	13.69	38.69	782	349	263	14.38 ± 2.68	23.20
	1st Pos.	28.83	17.60	23.44	30.12	260	79	47		
	2nd Pos.	21.63	22.19	14.99	41.11	261	35	11		
	3rd Pos.	40.67	12.19	2.17	44.96	261	235	205		

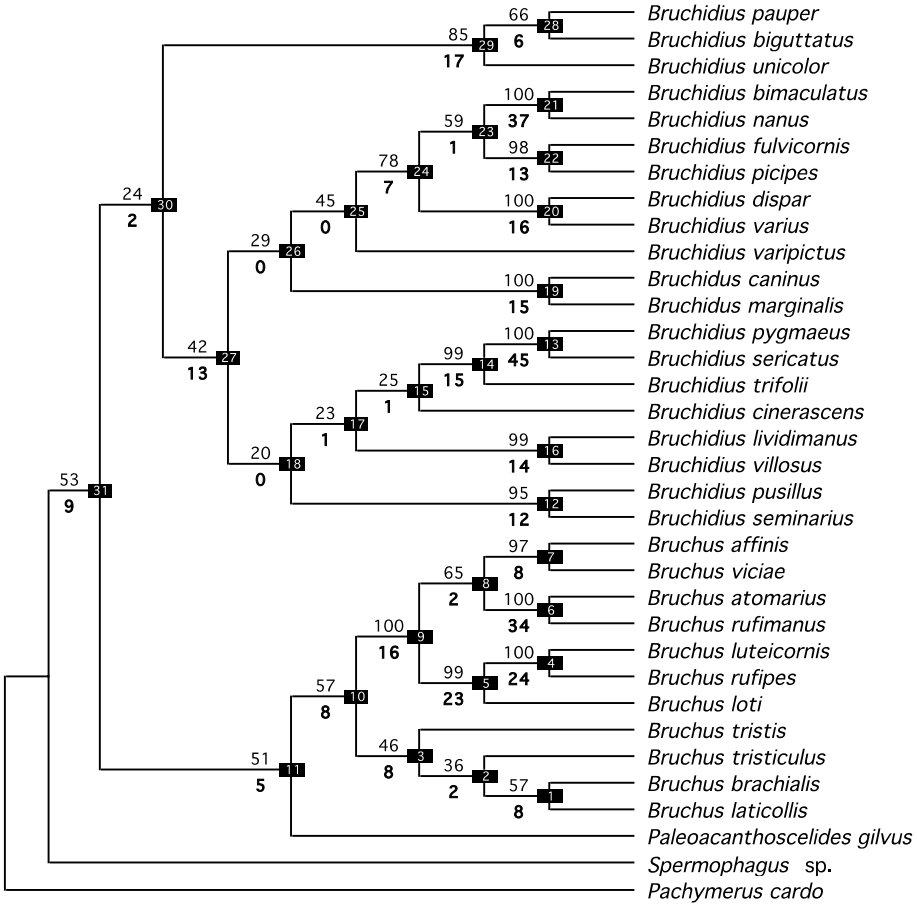

Fig. 1. Most parcimonious tree from the unweighted parsimony analysis of the combined data set. Numbers above branches are bootstrap support values and below are Bremer support values. Node numbers employed in Table 4 are labelled in black.

(Qiagen GmbH, Germany) and recovered in 40 μl elution buffer. Both strands of the PCR products were sequenced by the Sanger dideoxy method. Sequence data were obtained by analyzing samples on an ABI 373 Automated sequencer. We checked sense and antisense strands against each other. ClustalX (Thompson et al., 1997) was used for the 12S rRNA alignment under five different gap costs. Transitions and transversions were equally weighted, and with the exception of the default gap cost (opening cost 16, extension cost 6.66), we used the same cost for opening and extension gaps (2, 4, 8 or 16). In order to determine the 12S rRNA alignment for our analyses, we built an elision matrix (Wheeler et al., 1995) following Jordal et al. (2002). Afterwards, we retained the alignment yielding the most congruent consensus tree with the strict consensus tree from the elision matrix, i.e., the alignment corresponding to the default gap cost. Alignment of coding sequences (COI and Cyt *b*) was unambiguous and no gap event was detected. The sequences were all A + T biased, in agreement with previously published data on insect mitochondrial sequences (Clary and Wolstenholme, 1985; DeSalle et al., 1987). No significant base composition heterogeneity was detected between taxa for the 3 data sets (12S: $\chi^2 = 16.923739$, $df = 99$, $P = 1.000$; COI: $\chi^2 = 59.261822$, $df = 99$, $P = 0.99947552$; and Cyt *b*: $\chi^2 = 63.113383$, $df = 99$, $P = 0.99811916$). Table 3 summarizes the sequence variation observed in the three partial sequences obtained in this study. Of the 2206 nucleotide sites analysed, 39.52% were variable and about 30% were informative in parsimony analyses (this includes positions with gaps). For coding fragments, the majority of informative sites (80.99%) were located in third codon positions.

2.3. Phylogenetic analysis

Parsimony, maximum-likelihood (ML), and Bayesian inference (BI) were used to reconstruct phylogenetic relationships among taxa. Parsimony and ML analyses were performed using PAUP* version 4.0b10 (Swofford, 2002). Bayesian analyses were carried out by using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001). We used both Power Mac G4 867 MHz and Pentium IV 1.7 GHz for our analyses. Preliminary analysis of sequence data were performed by unweighted parsimony analysis for all gene data sets separately. All analyses used *Pachymerus cardo* as outgroup species. Congruence between the three gene data sets were assessed by the incongruence length difference test (ILD) (Farris et al., 1994) implemented in PAUP* 4.0b10 as the partition homogeneity test. Invariant characters were excluded from the data sets for the ILD test following Cunningham (1997). In addition, partitioned Bremer support indices (Baker and DeSalle, 1997; Baker et al., 1998) were calculated with TreeRot (Sorenson, 1999) to estimate the contribution of each data partition to nodal support. Modeltest 3.06 (Posada and Crandall, 1998) was used in order to determine the best-fit substitution model for the data under ML and BI through hierarchical likelihood ratio tests and the Akaike information criterion. Gaps constitute a valuable source of phylogenetic information (Giribet and Wheeler, 1999) and thus were treated as fifth character in the parsimony analyses, which were performed by using the heuristic search option with at least 100 random-addition replicates. Given the fact that transitions are more frequent than transversions, some authors have recommended to downweight transitions in parsimony analyses in order to minimize homoplasy (Hillis et al., 1994). Thus, we performed a weighted analysis of the combined data set in which transversions were weighted twice over transitions. Subsequently, we also performed two differential weightings of third codon positions (2:2:1 and 4:4:1) to minimize the effect of transitions that may accumulate at high frequency due to the degeneracy of the genetic code. Robustness of topologies was assessed by bootstrap procedures by using 100 replicates (full heuristic search) of 100 random-addition replicates each, for all analyses. Both likelihood ratio tests and Akaike

Table 4
Partitioned Bremer support indices for the three data partitions

Node	Cyt *b*	COI	12S rRNA	All
1	6.5	−3	4.5	8
2	2.5	−1	0.5	2
3	3.5	1	3.5	8
4	0	21	3	24
5	7	15.5	0.5	23
6	8.5	24.5	1	34
7	4	2	2	8
8	−6	4	4	2
9	4.8	7	4.2	16
10	11	−8	5	8
11	−1	3	3	5
12	2	5	5	12
13	13	26	6	45
14	0	12	3	15
15	0	3	−2	1
16	−11	13	12	14
17	−2	2	1	1
18	−3	1	2	0
19	4	6	5	15
20	−3	17	2	16
21	20	13	4	37
22	3	11	−1	13
23	0	−1	2	1
24	−2	6	3	7
25	−3	1	2	0
26	−3	1	2	0
27	4	3	6	13
28	10	0	−4	6
29	−6	12	11	17
30	1	0.5	0.5	2
31	3	1	5	9
Total	67.8 (18.72%)	198.5 (54.83%)	95.7 (26.43%)	362

information criterion selected the general time reversible model including the proportion of invariable sites and gamma distribution for rates variation among sites (GTR + I + Γ: Gu et al., 1995; Lanave et al., 1984; Yang, 1994) as the best-fit model for ML and BI phylogenetic analyses of the combined data. This model does not account gaps, which are treated as missing data in ML and BI phylogenetic analyses. ML parameters were estimated by using the topology resulting from the unweighted parsimony analysis and several iterations were performed to optimize them, until we obtained a stable topology. Then ML analysis was performed using the heuristic search option under PAUP* with 10 random-addition replicates. Robustness of ML topology was assessed by a ML bootstrap analysis (100 replicates, full heuristic search, same parameters), which took about a week to perform. For the Bayesian analysis, we performed a 2,000,000 generations run by using the MCMC algorithm implemented in MrBayes with four incrementally heated chains and parameters estimated with a GTR + I + Γ model. A burn-in period of 20,000 generations was identified graphically by plotting likelihood values for each generation. The results were presented in the form of a 50% majority-rule consensus tree and the support for the nodes of the tree were given by posterior probabilities of clades (clade credibility values). Using the character tracing option (default settings) in MacClade 4.05, we mapped the host-plant preference on the phylogenetic tree from the ML analysis.

3. Results

According to the ILD test, partitions of the data into 12S rRNA, COI, and Cyt *b* were homogeneous ($P > 0.05$). Thus, we combined both data sets for the phylogenetic analyses shown in this study. Unweighted parsimony analysis of the combined data set yielded two optimal trees of 4039 steps (CI = 0.336; RI = 0.417; and RCI = 0.140). Weighted parsimony analyses of the combined data set produced single tree of 5754 steps for the analysis in which we weighted transversions

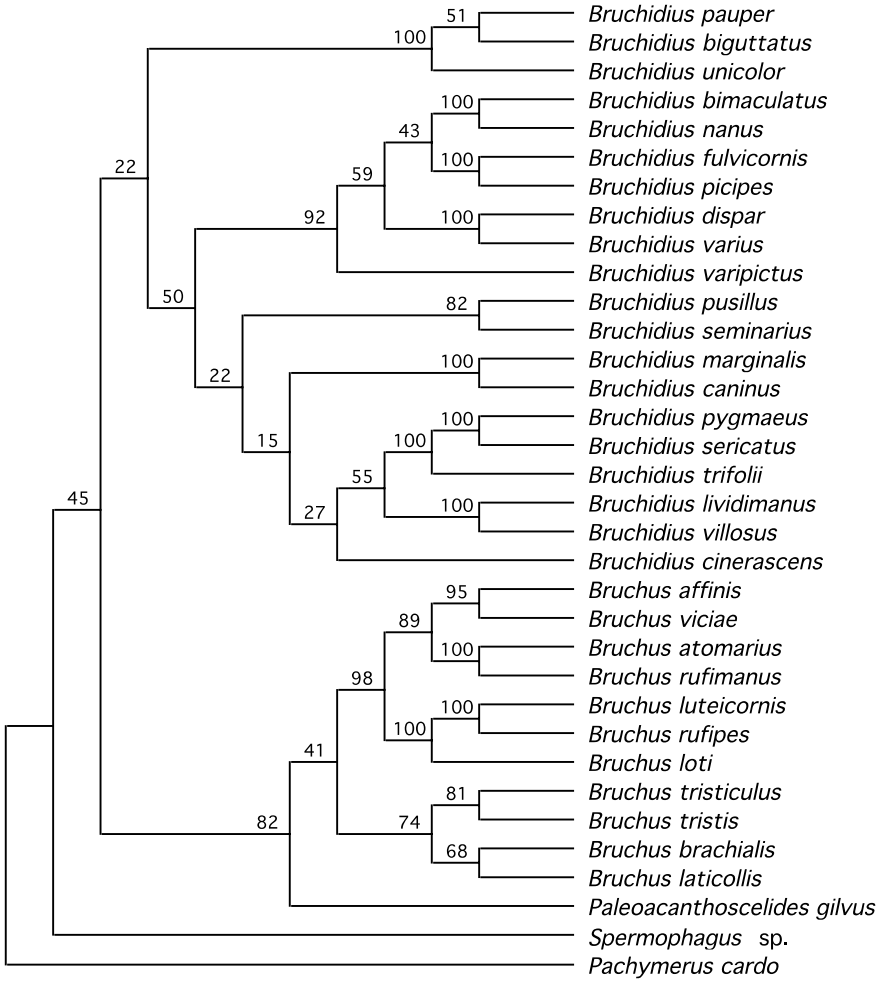

Fig. 2. Phylogenetic tree from the maximum likelihood analysis of the combined data set under the GTR + I + Γ model. Numbers above branches show the bootstrap support values.

twice over transitions (CI = 0.283, RI = 0.443, and RCI = 0.142). As regards the analyses with third codon position downweighted, the first (with 2:2:1 weighting scheme) yielded a 5089 steps tree (CI = 0.354, RI = 0.441, and RCI = 0.156) and the second (with 4:4:1 weighting scheme) produced a 7183 steps tree (CI = 0.374, RI = 0.468, and RCI = 0.175). The proportion of replicates which yielded optimal trees was of 80% on average for weighted parsimony analyses and 54.3 and 29.5% for the two trees resulting from the unweighted parsimony analyses. Unweighted and weighted parsimony analyses gave essentially the same topologies and for the sake of simplicity, we only present the optimal phylogenetic tree corresponding to the unweighted parsimony analysis (Fig. 1). Partitioned Bremer support indices (Table 4) were calculated using this topology and for the three data partitions, these indices are largely positive and thus support the combined analysis strategy. 54.83% of overall the Bremer support was from COI data, 26.43 from 12S rRNA data, and 18.72% from Cyt *b* data. Monophyly of the genus *Bruchus* was supported in all parsimony analyses (boostrap values >50%, Bremer support 8) whereas monophyly of the genus *Bruchidius* was poorly supported not only by bootstrap values in both unweighted and weighted parsimony analyses (25% on average) but also by a low Bremer support (2), although all partitions had a positive contribution to this node (Table 4). On the contrary, the genus *Bruchus* shows conflict in support between the Cyt *b* and 12S partitions (11 and 5, respectively), and the COI partition (−8) (Table 4). Weighted parsimony analyses yielded slightly higher support for basal nodes. These relatively low values contrasted with the well-supported relationships observed within the *Bruchus* clade we analysed. For the *Bruchidius* species it appeared that several basal nodes were poorly supported by bootstrap and Bremer support values but overall relationships between *Bruchidius* were well resolved. ML (Fig. 2) and BI (Fig. 3) phylogenetic analyses resulted in similar topologies. For ML, nine of the ten replicates yielded trees of optimal likelihood value. Both ML and BI analyses recovered the monophyly of the two genera.

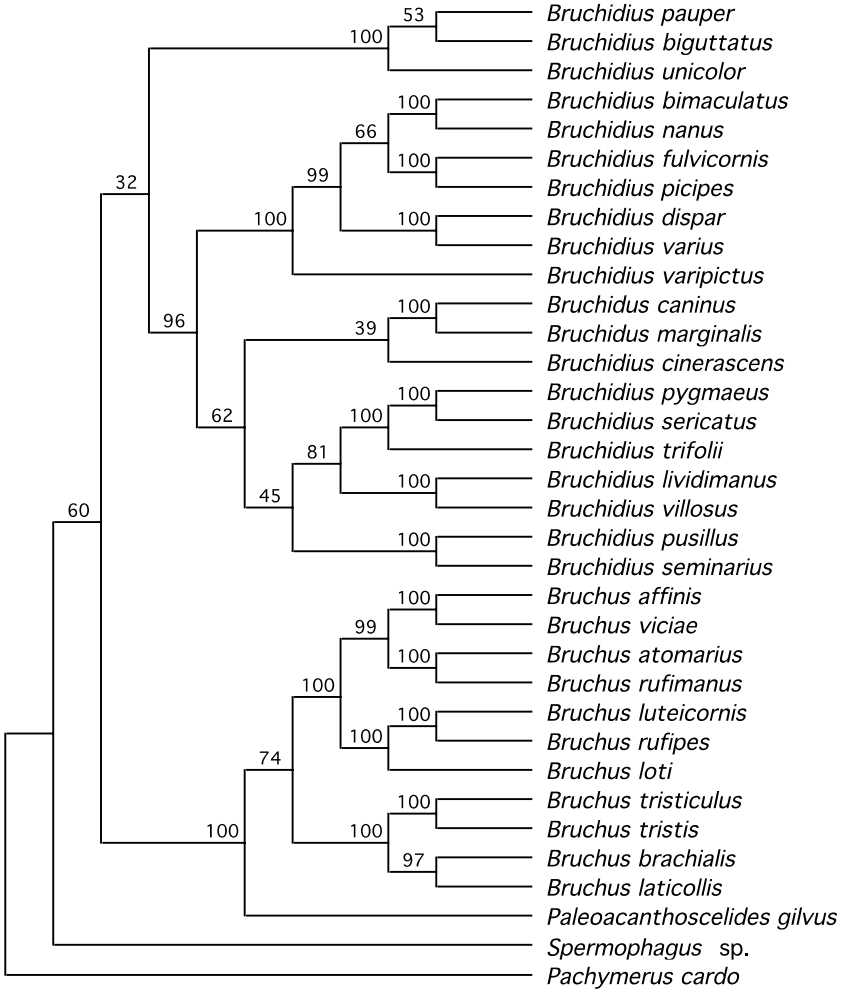

Fig. 3. Fifty percentage of majority-rule consensus tree from the Bayesian inference analysis of the combined data set under the GTR + I + Γ model. Numbers above branches show the posterior probability values.

The monophyly of *Bruchidius* was poorly supported by the bootstrap value (22%) and by the posterior probability (32%) in the BI analysis. Higher support was found for the monophyly of *Bruchus* bootstrap (41%) and posterior probability values (74%). Topologies obtained in the parsimony analyses were mostly congruent with the two topologies obtained from ML and BI analyses. Parsimony, ML, and BI recovered the same phylogenetic relationships for *Bruchus* species with the exception of *Bruchus tristiculus* and *Bruchus tristis*. These two species form a monophyletic group in all analyses except for the unweighted parsimony analysis. Several differences were observed in the *Bruchidius* topology between parsimony and ML/BI. The bootstrap support values (parsimony and ML) were low or moderate for most basal nodes but several internal nodes are well supported. By contrast, the overall level support of the BI topology was greater than bootstrap support of parsimony and ML analyses though we used the same model and parameters in BI and ML, as previously observed by Huelsenbeck et al. (2002) and Miller et al. (2002). Recent studies suggest that Bayesian posterior probabilities should overestimate phylogenetic support (Cummings et al., 2003; Douady et al., 2003; Suzuki et al., 2002). Caution must therefore be exerted when considering these values.

4. Discussion

All analyses support the monophyly of *Bruchus* and *Bruchidius* and figure *Paleoacanthoscelides gilvus* as the sister-group of the *Bruchus* group. Although these clades were moderatly supported by boostrap support values and Bremer indices nonetheless they were stable to parameter variation. This observation is consistent with a recent review (Giribet, 2003) showing evidence for nodal stability despite low nodal support. The phylogenetic relationship between the 11 *Bruchus* species in our sample is well resolved and presents essentially the same topology under all phylogenetic algorithms used. Relationships within the *Bruchidius* clade are mostly resolved but the position of *B. cinerascens* and that of the clade composed of *B. caninus* and *B. marginalis* remain unstable. Our results offer the opportunity to review existing taxonomic groups (Table 5) established by Borowiec (1988), primarily on the basis of external morphology. The monophyly of seven taxonomic groups (counting more than one species in our sample) recognized by Borowiec was supported by our analyses: the *affinis* group, the *atomarius* group, the *bimaculatus* group, the *brachialis* group, the *foveolatus* group the *rufipes* group and the *tristis* group. Two groups appear as paraphyletic in our analyses: the *seminarius* group and the *varius* group. These discrepancies between our molecular based results and Borowiec hypotheses help us reconsider some morphological characters used to define group boundaries. Species belonging to the *bimaculatus* and *varius* groups form a monophyletic clade in our analyses and are also well characterized by the particular shape of their male genitalia. So, the positioning of these species in two distinct groups apparently does not seem justified any longer. In the *seminarius* group, the shape of sclerites present in the median lobe (a structure of the male genitalia) seems to be a significant character distinguishing the two clades of this paraphyletic group. Consequently, this group could be separated into two distinct ones. Moreover, the study of some neglected morphological characters such as the shape of the urosternite (a structure of the *Bruchus* male genitalia) appears promising in view of some preliminary observations on our sample of *Bruchus* species. A critical analysis based on published data and field observations (Delobel and Delobel, 2003) enables us to define accurately the host-tribe or host-family (for non legume-feeders) of species included in this study. We mapped the hosts tribes and families on the phylogeny (Fig. 4) including new field observations (Delobel and Delobel, 2003) on the number and genera of host-plant species in order to make more precise their degree of specialization. As stated by Borowiec (1987), *Bruchus* species exhibit a significant level of specificity and are strictly associated with the tribe Vicieae in the Fabaceae,

Table 5
Taxonomic groups present in our sample according to Borowiec (1987)

Genus	Group	Species
Bruchidius	*bimaculatus*	*bimaculatus*
		nanus
	cinerascens	*cinerascens*
	foveolatus	*pygmaeus*
		sericatus
		trifolii
	pauper	*pauper*
	seminarius	*lividimanus*
		pusillus
		seminarius
		villosus
	unicolor	*unicolor*
	varius	*dispar*
		fulvicornis
		picipes
		pr. varius
Bruchus	*affinis*	*affinis*
		viciae
	atomarius	*atomarius*
		rufimanus
	brachialis	*brachialis*
		laticollis
	loti	*loti*
	rufipes	*luteicornis*
		rufipes
	tristis	*tristiculus*
		tristis

and all *Bruchus* species of our sample are equally associated with the genera *Lathyrus* and *Vicia*. For the *Bruchidius* species studied here it is difficult to distinguish a strong trend towards oligophagy (and even monophagy) or polyphagy. Yet the four most polyphagous species (*B. lividimanus*, *B. pusillus*, *B. seminarius*, and *B. villosus*) did not form a monophyletic group in our analyses. If we consider *Bruchidius* species, we notice that phylogenetically related insects feed on the same host-plant tribes, and in some cases of the same genus, especially in the case of the clade including the *bimaculatus* and *varius* groups. However, the insect–host plant pattern does not follow the host-plant phylogeny recently proposed by Wink and Mohamed (2003). Species of *Bruchidius* associated with Galegeae form a monophyletic group, and it is also the case of the two species associated with Genisteae. The most striking pattern apparent from Fig. 4 is the position of the Trifolieae feeders, which form two distinct monophyletic groups. This implies two independent host-shifts towards Trifolieae. This assumption is consistent with observations on the male genitalia, that show two distinct types. A similar host-shift pattern is observed in species associated with the Loteae tribe (*B. pauper*, *B. pusillus*, and *B. seminarius*). The fact that *B. pauper* appears as a very specialized species and *B. pusillus* and *B. seminarius* as highly polyphagous species suggests that ancestry or driving evolutionary mechanism of each Loteae shift could have been different. The analysis of the position of *B. biguttatus* is interesting: this species is morphologically very close to *B. pauper* and *B. unicolor* but it develops on plants belonging to a botanical family (Cistaceae) not phylogenetically related to Fabaceae. It likely experienced a shift to Cisteaceae from a Papilionoideae ancestral host-plant. A similar pattern can be observed for *B. cinerascens* (associated with Apiaceae) although its phylogenetic relationships with the other *Bruchidius* species remain uncertain.

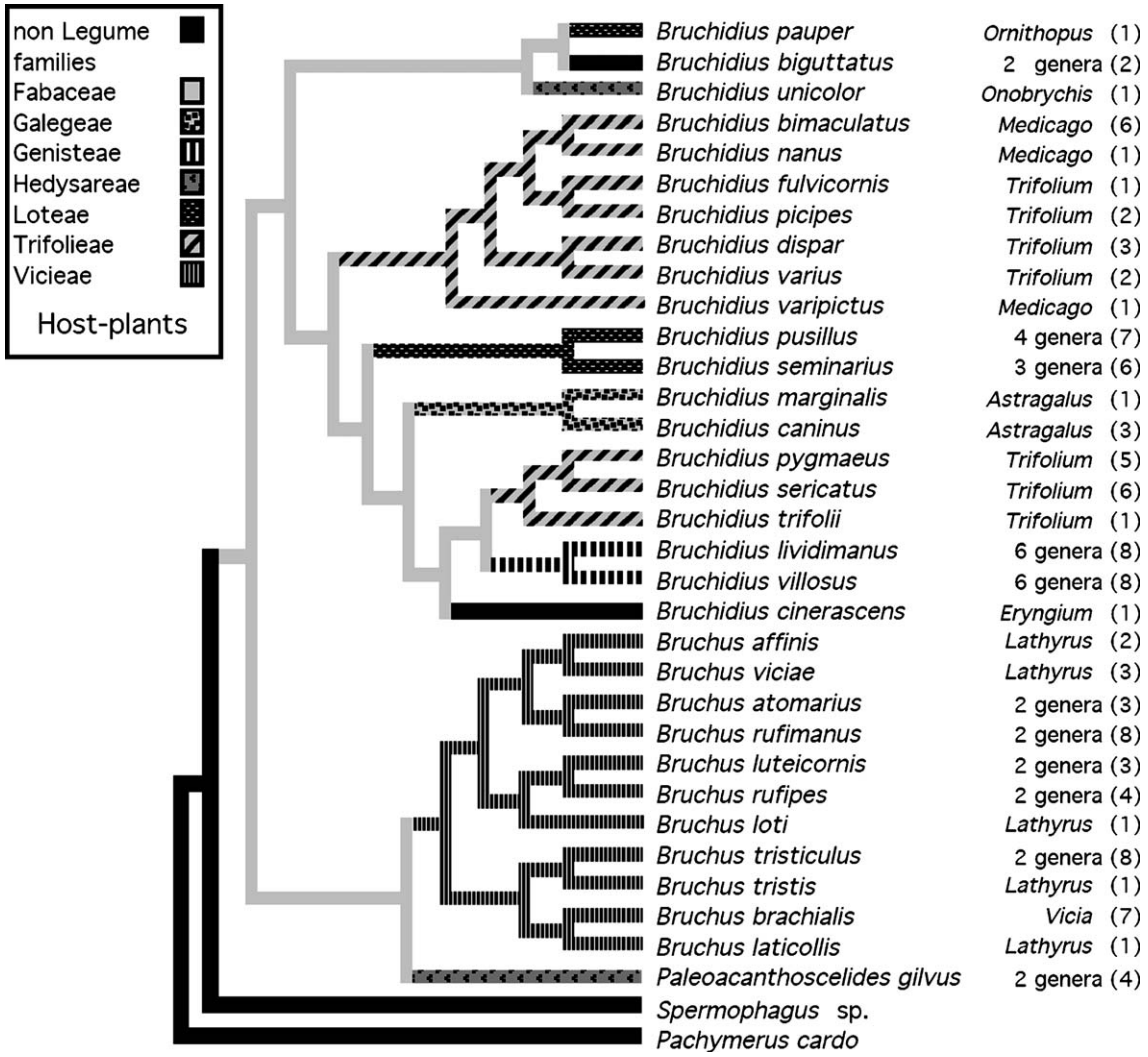

Fig. 4. Character tracing of host-plant families or tribes (for the family Fabaceae) on the phylogenetic tree from the maximum likelihood analysis. The host-plant genus or the number of host-plants genera (if the species feeds on different genera) is given for each bruchid species. The number of host-plants species for each bruchid species (see Delobel and Delobel, 2003 for details) is indicated in parentheses.

5. Conclusions

Our data provide the first well-supported phylogenetic hypothesis for a number of European bruchids and fill a gap emphasized by recent authors (Jermy and Szentesi, 2003). Topologies obtained support the monophyly of the genera *Bruchus* and *Bruchidius* with some reservations for the latter due to low nodal support, although the result is stable to parameter variation. The lack of resolution observed for some basal nodes may reflect an episode of rapid diversification among Bruchidae but this situation could also be explained by a lack of resolution at that level using our markers, or may be due to a sample bias. Our results indicate that phylogenetically related bruchids are generally associated with phylogenetically related host-plants of the same tribe. Nevertheless, we have no evidence of congruent phylogenies. Thus, it seems difficult to conclude on the exact nature of mechanisms which might explain the diversification of these insects. The inclusion of additional data on host-plant phylogeny and the nature of seed secondary compounds is a stimulating research perspective in the light of previous studies (Becerra, 1997; Farrell and Mitter, 1990; Silvain and Delobel, 1998; Termonia et al., 2001; Wahlberg, 2001; Wilf et al., 2000). Also, we should not neglect the contribution of morphological data to these studies, in particular those concerning the structure of the male genitalia which seems very informative. Understanding the evolution of the Bruchidae requires the integration of all possible data and well-founded phylogenetic hypotheses. This study should help us to shed new light on the evolution of plant–insect interactions.

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